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HUMAN IMMUNE RESPONSE TO BOTULINUM PENTAVALENT (ABCDE) TOXOID, DETERMINED BY A NEUTRALIZATION TEST AND BY AN ELISA.

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To determine the immune status of persons receiving Botulinum Pentavalent (ABCDE) Toxoid and to evaluate the effectiveness of the vaccine, we surveyed immunized individuals for neutralizing antibodies to type A and to type B botulinum toxins. After the primary series of three immunizations administered at 0, 2, and 12 weeks, 21 of 23 persons tested (91%) had a titer for type A that was $\geq$ 0.08 International Units (IU)/ml, and 18 (78%) had a titer for type B $\geq$ 0.02 IU/ml. (One International Unit is defined as the amount of antibody neutralizing 10,000 mouse median lethal doses of type A or B botulinum toxin). Just prior to the first annual booster, 10 of 21 people (48%) and 14 of 21 (67%) lacked a detectable titer for type A and for type B, respectively. After the first booster, all individuals tested had a demonstrable titer to both types A and B. Of 77 persons who had previously received from one to eight boosts of the toxoid, 74 (96%) had an A titer $\geq$ 0.25 IU/ml, and would not require an additional booster, according to the recommendations of the Centers for Disease Control. However, only 44 of 77 (57%) had a B titer $\geq$ 0.25 IU/ml. In

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(#19) each group by booster number, even the group having had eight boosts, at least one person would require re-immunization on the basis of B titer. There was a wide range of antibody levels among individuals at the same point in the immunization scheme. Results from an ELISA, with purified type A or type B neurotoxin as the capture antigen, were compared to neutralization test results on 186 serum samples for type A and 168 sera for type B. Statistically, the correlation coefficients for results from the two assays were high ($r=0.69$, $P < 0.0001$, for type A; and $r=0.77$, $P < 0.0001$, for type B). However, due to the wide dipersion of values obtained, using ELISA test results to predict neutralizing antibody levels is unwarranted.

Human Immune Response to Botulinum Pentavalent (ABCDE) Toxoid, Determined by a Neutralization Test and by an ELISA

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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ABSTRACT

To determine the immune status of persons receiving Botulinum Pentavalent (ABCDE) Toxoid and to evaluate the effectiveness of the vaccine, we surveyed immunized individuals for neutralizing antibodies to type A and to type B botulinum toxins. After the primary series of three immunizations administered at 0, 2, and 12 weeks, 21 of 23 persons tested (91%) had a titer for type A that was ≥ 0.08 International Units (IU)/ml, and 18 (78%) had a titer for type B ≥ 0.02 IU/ml. (One International Unit is defined as the amount of antibody neutralizing 10,000 mouse median lethal doses of type A or B botulinum toxin.) Just prior to the first annual booster, 10 of 21 people (48%) and 14 of 21 (67%) lacked a detectable titer for type A and for type B, respectively. After the first booster, all individuals tested had a demonstrable titer to both types A and B. Of 77 persons who had previously received from one to eight boosts of the toxoid, 74 (96%) had an A titer ≥ 0.25 IU/ml, and would not require an additional booster, according to the recommendations of the Centers for Disease Control. However, only 44 of 77 (57%) had a B titer ≥ 0.25 IU/ml. In each group by booster number, even the group having had eight boosts, at least one person would require reimmunization on the basis of B titer. There was a wide range of antibody levels among individuals at the same point in the immunization scheme. Results from an ELISA, with purified type A or type B neurotoxin as the capture antigen, were compared to neutralization test results on 186 serum samples for type A and 168 sera for type B. Statistically, the correlation coefficients for results from the two assays were high ($r = 0.69$, $P < 0.0001$, for type A; and $r = 0.77$, $P < 0.0001$, for type B). However, due to the wide dispersion of values obtained, using ELISA test results to predict neutralizing antibody levels is unwarranted.

INTRODUCTION

For more than 40 years, immunization with botulinum toxoid has been used to protect laboratory workers at risk for botulism due to contact with toxins produced by Clostridium botulinum. During World War II, a monovalent type A and a monovalent type B toxoid, both fluid and alum-adsorbed, were used to protect laboratory personnel (16). The toxoids yielded satisfactory immunity, as evidenced by the production of toxin-neutralizing antibodies. Later, the alum-adsorbed products were combined into a bivalent toxoid, and more than 1,100 injections of this material were administered (16). However, the antigens used were relatively crude. To reduce the rate of undesirable local and systemic reactions, a more purified bivalent AB toxoid was produced and tested (10). A pentavalent (ABCDE) toxoid, manufactured by Parke, Davis and Company (PDC) in 1958, was used to immunize approximately 400 people during clinical testing (9). The product contains formalin-inactivated botulinum toxins of types A, B, C, D, and E, adsorbed to aluminum phosphate, with thimerosal added as a preservative (8, 9). The preparation is relatively impure, containing only about 10% neurotoxoid for type A (2), and similar values are to be expected for the other types. The PDC product was distributed for human immunization until November of 1981. From 1970 to 1981, more than 1,600 persons received over 6,000 doses of the vaccine (8). The product is believed to have protected vaccinated individuals from botulism after laboratory accidents involving exposure to toxin via aspiration, inhalation, and skin contact (8).

The toxoid currently distributed by the Centers for Disease Control (CDC), prepared by the same methodology as employed by PDC, was manufactured by the Michigan Department of Public Health (MDPH). The human response to two lots of the MDPH product was significantly greater than to the PDC product for the type

B component, but the responses to types A and E did not differ (12 persons per toxoid) (1).

The standard test to determine antibody to botulinum toxin is the neutralization test, a mouse bioassay. In 1982, an enzyme-linked immunosorbent assay (ELISA), with monovalent A, B, or E or pentavalent toxoid as the capture antigen, demonstrated antibodies to botulinum toxins in the sera of two patients with infant botulism (17). A double-antibody sandwich ELISA was used to investigate the kinetics of one individual's immune respone to botulinum toxoid (7). This ELISA employed an unusual reagent, antitoxin prepared in immunologically tolerant rabbits (6), and crude type A toxin to detect serum antibody. Shone et al. (19) used purified neurotoxin as the capture antigen in an ELISA to determine serum antibody in 10 persons receiving botulinum toxoid, and compared ELISA values to neutralization titers.

In the study reported here, we surveyed personnel immunized with the MDPH botulinum toxoid for neutralizing antibodies to type A and to type B botulinum toxins. The response to type A has been shown to correlate well with the responses to types C, D, and E; typically the response to type B is the poorest (5, 9). The purposes of this research were to determine the immune status of personnel receiving the toxoid and to evaluate the effectiveness of the current vaccine. We describe an ELISA, with purified type A or type B neurotoxin as the capture antigen, and compare ELISA and neutralization test results on 186 serum samples for type A and 168 sera for type B.

MATERIALS AND METHODS

Vaccine. Botulinum Pentavalent (ABCDE) Toxoid was produced by MDPH in 1969-1971, and bottled under contract to the U.S. Army in 1978. Lot A-2, manufactured to contain less residual formaldehyde (0.022% compared to 0.034% in the PDC toxoid), was used to immunize personnel considered to be at risk for botulism at this laboratory. The primary series of immunizations consists of three deep subcutaneous injections of 0.5 ml each, administered at 0, 2, and 12 weeks, with the third immunization given 10 weeks after the second. The initial booster (0.5 ml, injected deep subcutaneously) is given 12 months after the first immunization of the primary series, and additional boosters are administered annually.

Human sera. Serum samples were received on a voluntary basis from employees who were being immunized with the MDPH product. Sera were not collected prior to the first immunization of the primary series, as previous investigators did not detect neutralizing antibodies to botulinum toxins in any of the approximately 500 such samples tested (16). These results were later confirmed for 50 preimmunization sera (10). In our study, serum was obtained approximately 14 days after the third injection for 23 persons completing the primary series of immunizations. Paired sera were assayed for 98 people who had been on the immunization schedule for varying periods of time; sera were collected just prior to the toxoid injection and at some period after the immunization, usually two weeks. Aliquots of the sera were prepared and stored at -70°C until assayed. Prior to assay in the ELISA, all sera were incubated at 56°C for 30 min to inactivate complement and eliminate interference from complement-mediated reactions.

Neutralization test. Neutralizing antibodies to type A or to type B

botulinum toxin were determined by the mouse bioassay (12). Serum samples were serially diluted by fourfold, and five dilutions (1/4 to 1/1024) were tested to obtain an endpoint. The concentration of neutralizing antibodies in the serum was calculated relative to a World Health Organization Standard Antitoxin (equine) which was included in each test, and results are reported as International Units/ml. [One International Unit (IU) is defined as the amount of antibody neutralizing 10,000 mouse intraperitoneal median lethal doses (LD_{50}) of type A, B, C or D botulinum toxin or 1,000 mouse intraperitoneal LD_{50} of type E (3).] Sera that did not protect mice from death at a 1/4 dilution are reported as < 0.08 IU/ml for type A or < 0.02 IU/ml for type B; sera that protected all mice at a 1/1024 dilution were retested at higher dilutions.

ELISA. The purified type A or type B neurotoxin used as the capture antigen in the ELISA was prepared by minor modification of the methods previously described for type E (18). Results obtained from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (13) indicated that each neurotoxin was ≥ 95 % pure. The optimum concentrations of the neurotoxins and the reference positive standard (human botulism immune globulin) for use in the ELISA were determined experimentally by checkerboard titrations. For the assay of test sera, microtiter plates (96-well, flat-bottom, Immunlon™ 2; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 100 µl of purified type A or type B neurotoxin per well, diluted to approximately 5 µg/ml in coating buffer (0.05 M sodium carbonate/bicarbonate buffer, pH 9.6). The plates were incubated at 4°C overnight in a sealed plastic bag to prevent drying, and washed four times with phosphate-buffered saline (PBS; 0.01 M phosphate, pH 7.4, plus 0.86% NaCl) containing 0.1% Tween 20 (200 µl/well). Unbound sites on the well were blocked

by the addition of 200 µl of 1.0% bovine serum albumin in PBS to all wells. After incubation for 1 h at 37°C, each plate was washed four times, as described above. Test and control sera were prediluted 1/20 in PBS-Tween 20 (wash buffer) and 200 µl was added to the top row of the plate. Each serum was serially diluted twofold by transferring 100 µl into an equal volume of wash buffer in the next well down the column. Thus, dilutions of serum from 1/20 to 1/2,560 were tested. After incubation for 2 h at 37°C, the plates were washed and protein A-horseradish peroxidase conjugate (Sigma Chemical Co., St. Louis, Mo.), diluted to 1 µg/ml in wash buffer, was added (100µl/well). The plates were incubated for 30 min at room temperature, then washed. The substrate-chromagen mixture was prepared immediately before use by dissolving ABTS [2,2'-azino-bis(3-ethylbenzthiazolinesulfonic acid), diammonium salt; Sigma Chemical Co.] to a concentration of 1 mg/ml in substrate buffer (50 mM Na_2HPO_4 plus 25 mM citric acid, pH 5.0), and hydrogen peroxide was added to a final concentration of 0.03%. After the addition of the substrate-chromagen mixture (100 µl/well), the plates were incubated for 20 min at room temperature, and 50 µl of 3 N sulfuric acid per well was added. Absorbance at 414 nm (A_{414}) was measured immediately in a Titertek Multiscan ELISA plate reader (Flow Laboratories, Inc., McLean, Va.). Each microtiter plate contained a reagent blank, human botulism immune globulin as a reference standard, and normal human serum (Fisher Scientific Co., Orangeburg, N.Y.) as a negative control. All test sera were assayed in duplicate and paired sera were assayed on the same plate.

Statistical analyses of ELISA data. ELISA data were analyzed by the method of Manclark et al. (14). The mean A_{414} for duplicate samples was plotted versus the $\log_{10}$ dilution. Using the linear region of the curves, the slope of the

titration plot of the test sample was compared to that of the reference standard on the same plate. If the lines were parallel, the antitoxin content of the serum was calculated and expressed as relative potency (unknown/reference standard). If the lines were not parallel, (sera that were low in potency relative to the standard) the absorbance of the initial dilution was used to calculate antitoxin levels.

RESULTS

Toxin-neutralizing antibody. The serological responses to the A and B components of the pentavalent toxoid after the primary series of immunizations are shown in Table 1. Serum samples were obtained 13 to 22 days following the third (or fourth, in one case) injection of toxoid. Of the 23 individuals receiving the primary series, only one did not have a detectable titer against either type A or type B toxin. One other person had an undetectable titer for type A, but had a demonstrable titer for type B, while four individuals had a titer of < 0.02 IU/ml for B, but had a measurable titer for A. The person with the greatest response to the toxoid after the primary series was immunized at 0, 15, and 25 weeks. Even excluding the data from this individual, there was a wide range of antibody levels for each type, more than 20-fold for A, and more than 50-fold for B.

Immediately prior to the administration of the first annual booster, 10 of the 21 individuals tested did not have a measurable antibody titer for type A and 14 lacked a demonstrable titer for type B (Table 2). These individuals were not those who were tested after the primary series, as the time frame of this study precluded sequential samples from the same individual to monitor immune status over time. Approximately 2 weeks after the first annual booster (range of 7 to 43 days), a second blood sample was drawn and assayed. The boost produced the expected increase in titer (Table 2).

The geometric mean titers pre- and post-boost for individuals grouped according to length of time on the immunization schedule are shown in Table 3. There was a wide range of antibody levels among individuals at the same point in the immunization scheme. As anticipated, a rise in titer occurred after each boost. For 220 serum samples assayed in both the A and B neutralization tests, 210 (95%) had an A titer greater than the B titer.

ELISA. Using an ELISA system with purified type A neurotoxin as the capture antigen, we assayed 186 sera and compared the results with the type A neutralization titers (Fig.1). In eight samples, antibody was not detected in the neutralization test (≤ 0.08 IU/ml) nor in the ELISA (relative potency < 0.01). Both tests were positive for 150 sera. Eight samples that were negative in the neutralization test did react in the ELISA, with the range of values from 0.045 to 0.22. However, two of those sera were drawn after the primary series, and 5 were obtained just prior to the first booster. Since these samples are from individuals just beginning the immunization program, antibody avidity is low, and the ELISA may be better able to detect such antibody than the neutralization test. There were 20 sera that had neutralizing antibody (range of 0.08 to 2.42 IU/ml), but did not react in the ELISA; two were after the primary series, and eight were prior to the first booster. Statistically, the correlation coefficient for 186 samples was good ($r = 0.69$, $P < 0.0001$). If the data are deleted for all sera drawn after the primary series and prior to the first boost, the correlation coefficient ($n = 144$) decreased to 0.66. Although the correlation between the two methods is statistically significant, it is of little practical value. Due to the dispersion of values shown in Fig. 1, the type A ELISA could not be used as a replacement for the neutralization test.

Similarly, we assayed 168 sera using an ELISA with purified type B neurotoxin as the capture antigen (Fig. 2). Antibody was not detected in the neutralization test (≤ 0.02 IU/ml) nor in the ELISA (relative potency ≤ 0.01) for seven samples. Both tests were positive for 148 sera. Three samples were positive in the neutralization test (0.03, 0.07, and 0.14 IU/ml), but did not react in the ELISA. Ten sera that were ELISA-positive did not have detectable neutralizing antibody; four of those were drawn after the primary series, and

five were drawn prior to the first boost. The range of ELISA values was 0.16 to 0.253 for 9 of the 10 samples; one serum, drawn after the primary series, had an ELISA value of 8.2. Again, the overall correlation coefficient (n=168) was good ($r=0.77$, $P < 0.0001$). Deleting the data for sera drawn after the primary series or before the first booster increased the correlation coefficient (n=133) to 0.80. However, as for type A, the correlation for type B is of little practical value.

The A and B ELISAs were quite reproducible, both between plates assayed on the same day and from one day to another, as indicated by results obtained with the reference positive standard.

DISCUSSION

The relationship between the concentration of serum antibody and the ability to resist the adverse effects of botulinum toxins is, of course, unknown for humans. "Satisfactory" levels of antitoxin have been chosen based on data obtained with experimental animals and extrapolated to man. Investigations with guinea pigs demonstrated that animals with serum antitoxin levels of approximately 0.02 units/ml could withstand challenge with 2×10^5 minimum lethal doses (MLD) of toxin, and that values of 0.1 to 0.5 units/ml were protective against up to 1×10^6 MLD of toxin administered parenterally (16). Based on these data, 0.02 unit/ml was believed to be a protective level in humans, and attaining that immunological response was the goal of the initial research on type A and type B botulinum toxoids (16). In a separate study, Fiock et al. (10) confirmed the earlier results correlating serum antibody and resistance to toxin challenge in guinea pigs. Since one unit of their type B antitoxin neutralized about four times as much homologous toxin as did a unit of type A antitoxin, they chose 0.005 unit/ml as a protective level for type B and continued to employ 0.02 unit/ml as the standard for type A (10). These values were also used in the evaluation of the pentavalent (ABCDE) toxoid (9), and are twice the lowest titer that can be determined using the mouse bioassay (5). However, guinea pigs with antitoxin levels that were undetectable in the neutralization test survived challenge with large doses of toxin, so levels that are not deemed "satisfactory" probably provide significant protection (10).

Fiock et al. (9) evaluated the human immune response to four lots of pentavalent toxoid produced by PDC. The percentage of recipients with measurable titers two weeks after completion of the primary series ranged from 65 to 97% for type A and 56 to 93 % for type B. In our study, 23 persons were

tested for their antibody response to the A and B components of the MDPH pentavalent toxoid approximately two weeks after receiving the third immunization of the primary series (Table 1). Only one individual had serum antibody levels for both A and B that were below the limits of detection of our assay. Twenty-one (91%) had a titer for type A that was ≥ 0.08 IU/ml, and 18 (78%) had a titer for type B ≥ 0.02 IU/ml. The highest titers were attained by an individual who received the primary series at 0, 15, and 25 weeks (Table 1). Although only one person was immunized by this unusual schedule, perhaps extending the timing of the immunizations of the primary series could result in increased protection. However, this is precluded by the need to achieve a protective level as quickly as possible, and a 25 week period, rather than 12, to complete the primary immunization series is not time efficient. The decline in antitoxin levels by 52 weeks and the effectiveness of the first booster (9, Table 2) suggest that a booster of toxoid at 6 months may be desirable. However, Fiock et al. (10) found that even though the pre-boost titers were essentially the same, antibody titers after a boost at 26 weeks were much lower than after a boost at 52 weeks. Therefore, the incorporation of an additional injection at 6 months into the immunization protocol may be advantageous. Antitoxin levels produced by such a schedule of immunizations remain to be determined.

The Centers for Disease Control recommends against a second or subsequent boost if an individual has a titer of 1:16 or greater, approximately 0.25 IU/ml of neutralizing antibody, for the toxin types to which he/she is at risk (8). Of the 77 individuals we tested who had received from one to eight boosts of the MDPH toxoid, 74 (96%) had an A titer ≥ 0.25 IU/ml, but only 44 (57%) had a B titer ≥ 0.25 IU/ml. In each group by booster number, even after eight boosts, at least one person would require reimmunization on the basis of their B titer. The data

presented in Tables 1, 2 and 3 demonstrate the wide range of antibody titers among individuals who received the same number of immunizations. Fiock et al. (9) also noted a 1,000-fold range of titers within a group of immunized individuals who had received the same vaccine according to the same schedule.

The neutralization test, a mouse lethality bioassay, has been the standard method to measure antibody to botulinum toxins for many years. There are several disadvantages to this procedure. It requires the use of experimental animals, which can be objectionable on the basis of cost and ethical concerns. The four day observation period for mouse lethality, as well as space limitations for animals, can adversely impact on the number of assays that can be completed. Furthermore, there are safety concerns due to the handling of toxin in syringes. An alternative test, the ELISA, has been described to quantitate antibody to botulinum toxins (7, 17, 19).

An ELISA, with type A, B, or E (each monovalent) or pentavalent toxoid as the capture antigen, was developed and used to measure serum antibody levels in two patients with infant botulism (17). Although laboratory results demonstrated that one case was caused by toxicoinfection with C. botulinum type A and one case by type B, each patient's serum reacted in both the A and B ELISA. Since the toxoids used as reagents in the assay are impure (2), this ELISA may measure antibody to the other components of the vaccine rather than to the inactivated toxin. Another ELISA, a double-antibody sandwich method, has been described and used to measure the kinetics of the immune response to the MDPH pentavalent toxoid in one person (7). This procedure used antibody from immunologically tolerant rabbits immunized with type A, but the rabbit serum also reacted with type B (6). Their ELISA detected antibody to components of the vaccine 15 weeks before neutralizing antibody could be detected in the mouse

bioassay (7). Each of these ELISAs measures antibody to components of the toxoid, but the immune status of an individual relative to the toxin would be difficult to ascertain from such assays. A good correlation between neutralizing activity and ELISA titer has been demonstrated for antibody to tetanus toxoid (15). Eighty serum samples, ranging from < 0.01 to >100 IU/ml, were assayed by both techniques. The ELISA used plates coated with tetanus toxoid. Rather than using botulinum toxoid in the ELISA, Shone et al. (19) employed purified type A or type B neurotoxin as the capture antigen. The results of the ELISA were compared to those of the neutralization test, for type A and for type B, for 10 human serum samples from immunized personnel. Values for each assay were tabulated and expressed as percentages of the value obtained with a pool of positive control sera. Results were correlated to a limited extent for high-titer sera, but those with lower titers gave ELISA readings that were barely above background.

We have used purified neurotoxin as the capture antigen in our ELISA, and compared results obtained to neutralization test results for 186 sera for type A (Fig. 1) and 168 sera for type B (Fig. 2). Since ELISA's that test only a single dilution of serum make the quantitation of antibody difficult (4), we used twofold serial dilutions of serum in our ELISA to compare the dose-response curve for the test sera with that of a standard antiserum assayed on the same plate. We employed human botulism immune globulin as the standard in the ELISA, because the W.H.O. Standard Antitoxin, used in the neutralization test, was produced in horses (3). The ELISA overcomes many of the disadvantages of the neutralization test: it does not require experimental animals, the test can be completed in about 7 h, and many samples can be assayed in 1 day. However, one disadvantage of our ELISA is the quantity of purified neurotoxin consumed. The

concentration used to coat the plates (5 µg neurotoxin/ml) was experimentally determined by checkerboard titrations, and was in the range of 1 to 10 µg/ml which is typically used for protein antigens. Thus, 45 µg of purified neurotoxin was required to assay four test sera in duplicate. In contrast, the neutralization test employed crude toxin, and 5 ng neurotoxin in an impure form was sufficient to assay 10 test sera.

Our ELISA measures antibody that will bind to any of the various antigenic determinants on the neurotoxin molecule, while the neutralization test measures antibody that abolishes the lethal biological activity of the neurotoxin. The correlation of ELISA test results with those obtained in the neutralization test is poor for serum samples from individuals early in the immunization series. (Deleting such data has a minimal effect on the correlation coefficient, however, since the number of samples is small relative to the total number of samples assayed.) Sera obtained after the primary series or prior to the first booster may have antibodies of low avidity, which are more reactive in the ELISA than in the neutralization test. Since antibody avidity increases with repeated immunizations, the correlation between ELISA and mouse bioassay results is higher for hyperimmune sera. Similar results have been reported for tetanus toxin (11). Thus, unless the origin of the serum sample is known, the usefulness of the ELISA to measure antibodies to botulinum neurotoxins would be limited. Even for hyperimmune sera, using ELISA results to estimate neutralization titer, and thus resistance to the adverse effects produced by botulinum toxin, is unwarranted, due to the wide range of values obtained (Fig. 1 and 2). Similarly, use of ELISA data to decide if an individual requires reimmunization would be ill-advised. Perhaps the ELISA could be used to monitor antibody levels in immunized personnel or to evaluate potential new vaccines or immunization

schedules, but results obtained cannot be extrapolated to toxin-neutralizing antibody levels.

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TABLE 1. Neutralization titers to types A and B botulinum toxins, after the primary series of immunizations, for 23 individuals.

A Neut. (IU/ml)	B Neut. (IU/ml)	Immunization Schedule (weeks)	Time[a] (days)
<0.08	<0.02	0-2-12	15
<0.08	0.03	0-2-12	14
0.07	0.03	0-2-12	14
0.09	<0.02	0-2-12	20
0.18	<0.02	0-4-14	14
0.18	0.15	0-7-16	15
0.25	<0.02	0-3-13	21
0.28	0.08	0-2-4-8	14
0.32	0.26	0-3-13	14
0.36	1.02	0-3-13	14
0.36	0.06	0-3-13	19
0.39	0.05	0-2-12	14
0.81	0.12	0-2-12	14
0.90	0.25	0-3-10	13
0.96	0.19	0-3-13	22
0.96	<0.02	0-3-13	15
0.99	0.12	0-3-13	14
1.08	0.07	0-4-14	15
1.28	0.06	0-3-13	18
1.28	0.19	0-2-12	16
1.71	0.14	0-3-13	14
1.81	0.33	0-3-13	14
14.0	0.30	0-15-25	15

[a] Time elapsed between the last immunization of the primary series and the date that the blood sample was drawn.

TABLE 2. Neutralization titers to types A and B botulinum toxins, before and after the first booster.

Pre-Boost		Post-Boost		
A Neut. (IU/ml)	B Neut. (IU/ml)	Time[a] (days)	A Neut. (IU/ml)	B Neut. (IU/ml)
<0.08	<0.02	15	0.40	0.05
<0.08	<0.02	7	0.48	0.16
<0.08	<0.02	14	1.10	0.76
<0.08	<0.02	14	5.12	0.75
<0.08	<0.02	14	14.5	1.89
<0.08	<0.02	14	26.6	2.56
<0.08	<0.02	16	3.23	0.64
<0.08	<0.02	18	3.62	2.18
<0.08	<0.02	43	17.2	0.61
<0.08	0.03	16	41.0	3.23
0.08	<0.02	15	5.12	1.94
0.09	<0.02	15	1.66	2.09
0.10	0.02	21	14.5	1.66
0.11	0.46	14	6.06	0.46
0.12	<0.02	28	8.80	2.87
0.16	<0.02	21	6.45	0.87
0.19	<0.02	15	36.5	2.56
0.20	0.16	15	12.9	0.81
0.51	0.04	14	10.2	0.60
1.23	0.04		Not Boosted	
2.03	0.15	19	8.13	2.18

[a] Time elapsed between the booster immunization and the date that the post-booster blood sample was drawn.

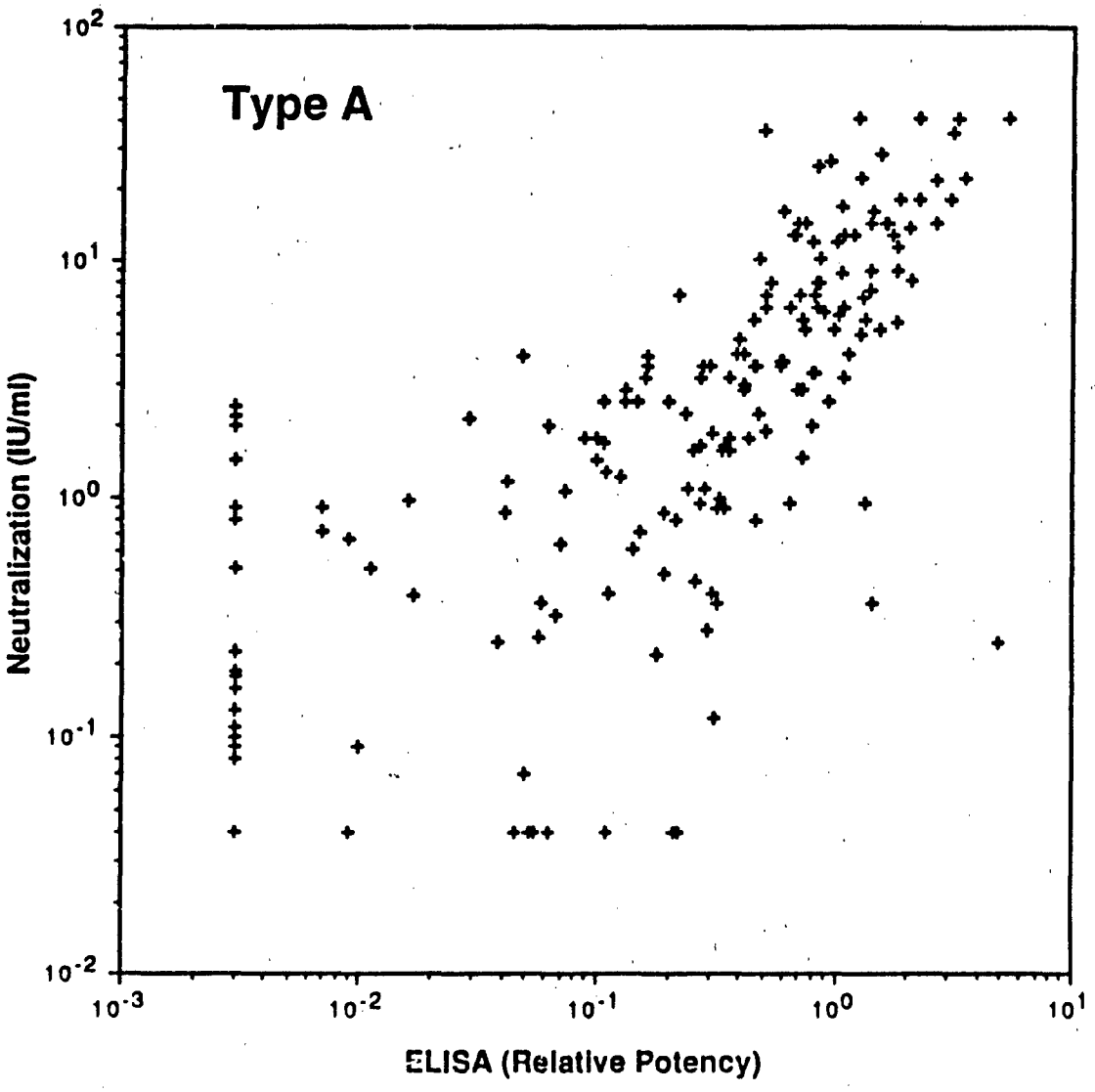
TABLE 3. Geometric mean titers (range) to types A and B botulinum toxins

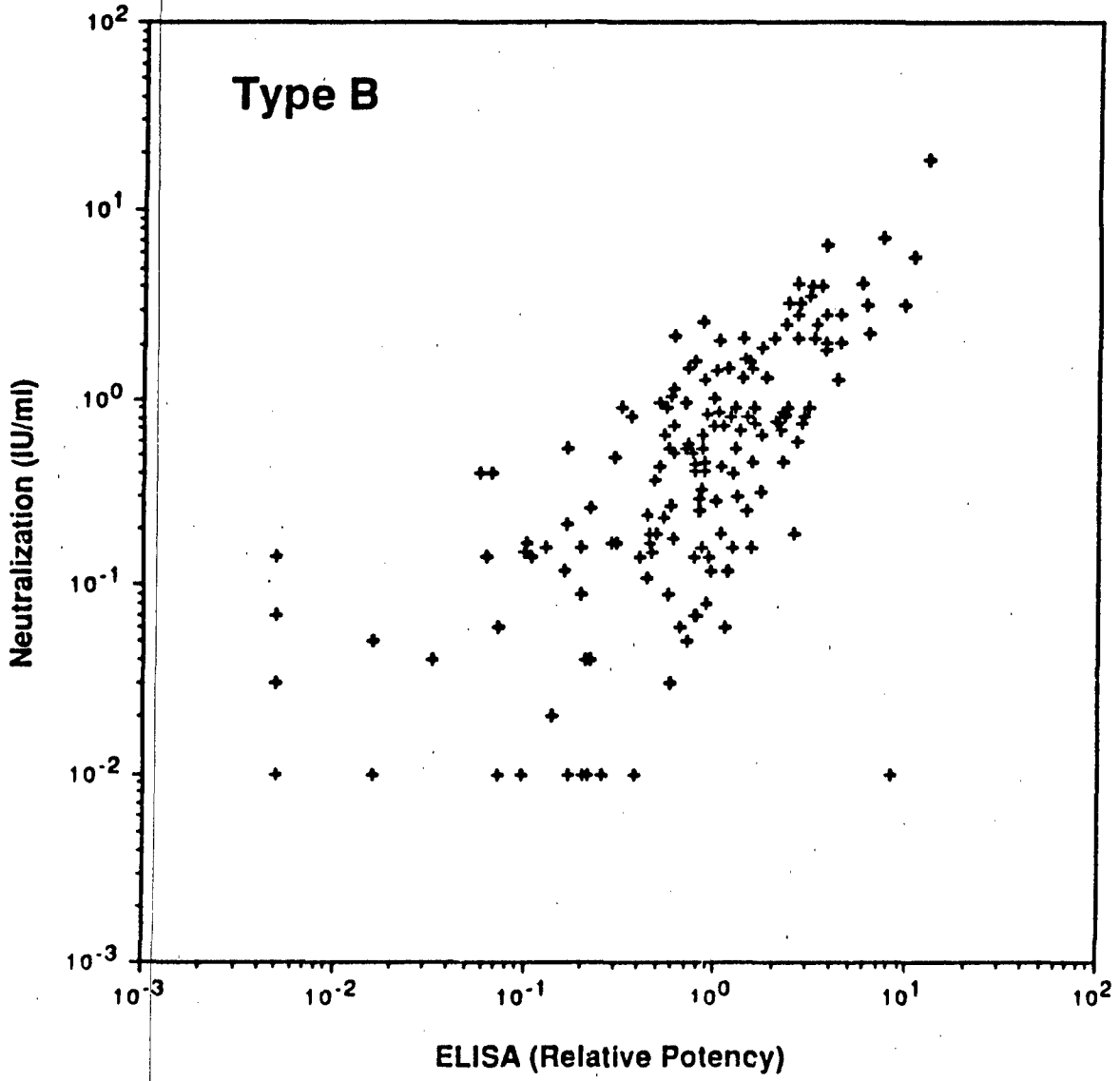
Booster Number	n	Pre- Boost A Neut (IU/ml)	Pre- Boost B Neut (IU/ml)	Post- Boost A Neut (IU/ml)	Post- Boost B Neut (IU/ml)
Primary Series	23	-	-	0.49 (<0.08-14.0)	0.08 (<0.02-1.02)
1	21	0.14 (<0.08-2.03)	0.03 (<0.02-0.46)	6.13 (0.40-41.0)	1.01 (0.05-3.23)
2	17	0.77 (<0.08-2.56)	0.20 (<0.02-0.72)	6.89 (0.40-35.2)	1.19 (0.12-4.18)
3	18	1.13 (0.23-10.2)	0.14 (<0.02-0.72)	5.70 (1.19-26.6)	1.12 (0.13-3.32)
4	9	1.52 (0.40-3.96)	0.26 (0.04-1.28)	7.01 (2.28-51.6)	1.54 (0.55-18.2)
5	13	1.50 (<0.08-4.77)	0.61 (0.07-3.62)	5.79 (0.91-23.0)	1.58 (0.55-7.24)
6	5	2.50 (0.72-4.06)	0.47 (0.09-0.91)	17.7 (3.96-41.0)	2.59 (0.81-6.64)
7	5	2.15 (<0.08-12.9)	0.31 (0.06-2.28)	6.75 (0.90-41.0)	1.53 (0.55-5.74)
8	5	2.82 (1.81-4.98)	0.41 (0.14-1.02)	12.8 (6.01-29.0)	1.55 (0.46-3.23)
9	5	4.23 (1.50-25.8)	0.31 (0.07-1.09)	17.2 (12.0-41.0)	0.78 (0.18-3.46)

FIGURE LEGENDS

FIG.1. Relationship between neutralization titer (IU/ml) and ELISA titer (relative potency) for type A for 186 human serum samples.

FIG. 2. Relationship between neutralization titer (IU/ml) and ELISA titer (relative potency) for type B for 168 human serum samples.


Type A
Neutralization (IU/ml)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
ELISA (Relative Potency)


Type B
Neutralization (IU/ml)
ELISA (Relative Potency)
10^2
10^1
10^0
10^-1
10^-2
10^-3
10^-3
10^-2
10^-1
10^0
10^1
10^2